FEEDING BIOLOGY OF *POLYMASTIA CROCEUS*

A.H BELL, P.R. BERGQUIST AND C.N. BATTERSHILL

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Polymastia croceus is a yellow, encrusting, marine sponge endemic to New Zealand's coastal waters. It is currently of great interest due to its production of a proteinaceous secondary metabolite which has potential for use in anti-cancer and anti-HIV pharmaceuticals. An examination of feeding in *P. croceus* was undertaken, to determine its importance in coastal ecology and implications for aquaculture, using *in situ* flow cytometry. High proportions of ultraplankton (cells <5μm) were consumed and *P. croceus* appeared to be selective in its feeding at one of the sites sampled. The ultraplankton species best retained were *Synechococcus*-type cyanobacteria (up to 94%) and picoeukaryotes (up to 88%), in contrast to previous studies where sponges were found to retain *Prochlorococcus* spp. most efficiently. Using a microthermistor-based flow meter, attempts were made to quantify the rate at which *P. croceus* processes water. From initial results *P. croceus* was shown to process large quantities of water at rates (up to 8.82cm^3 s^{-1}), well in excess of those previously recorded for other sponge species. These preliminary data indicate that *P. croceus* has potential to process large quantities of water in short periods of time. The highly efficient retention of ultraplankton species, together with the large volumes of water processed, indicate that sponges like *P. croceus* are likely to be a major component in the benthic-pelagic carbon cycle. *Polymastia croceus* is an abundant species and therefore likely to play a significant role in coastal foodwebs. Furthermore, we suggest that the contribution of sponges to coastal production deserves more attention. ❒ *Porifera, feeding biology, ultraplankton diet, water flow rates, Polymastia croceus.*

Andrew H. Bell (email: ahbell@clear.net.nz) & Patricia R. Bergquist, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand; Chris N. Battershill, Australian Institute of Marine Sciences, PMB 3, Townsville, Queensland, Australia; 23 April 1999.

Research on *in situ* pumping rates and diets of sponges has been minimal worldwide, with most effort attributed so far to Reiswig (1971-1974) and Pile et al. (1996, 1997). Nevertheless, these few studies show that sponges can process large amounts of water, extracting high percentages of the available plankton, in particular the fraction less than 10μm cell size. Pile et al. (1996) further defined the fraction of diet less than 10μm to show that there was high retention (>70%) of hetertrophic bacteria, *Prochlorococcus* spp., *Synechococcus*-type cyanobacteria, picoeukaryotes and nanoeukaryotes. These observations have implications for the distribution and abundance of sponges, and the marine ecosystems in which they exist. The high rates of feeding activity described in this literature indicate that sponges are important components of benthic-pelagic coupling.

Polymastia croceus is an abundant, yellow, encrusting, marine sponge, endemic to New Zealand's coastal waters. It has recently attracted interest due to its production of a proteinaceous secondary metabolite, which has potential for use in anti-cancer and anti-HIV pharmaceuticals. As the metabolite is present in sponges in only trace levels, alternative modes of production are being examined. Wild harvest and aquaculture are options for producing the large quantities of sponge biomass required to supply sufficient metabolite for continuing research. However, it is generally considered that harvesting the required biomass directly from wild stocks is unsustainable and thus artificial supply options have to be considered. Of these options, aquaculture appears to be the one most likely to be rapidly developed (Battershill & Page, 1996). Knowledge of the feeding biology is of fundamental importance to the design and implementation of any aquaculture regime, and the assessment of the impact of removal or addition of *P. croceus* to the ecosystem.

Previous research on *P. croceus* has been restricted to its reproductive ecology (Battershill & Bergquist, 1999a, in press) and taxonomy (Kelly-Borges & Bergquist, 1997). The work

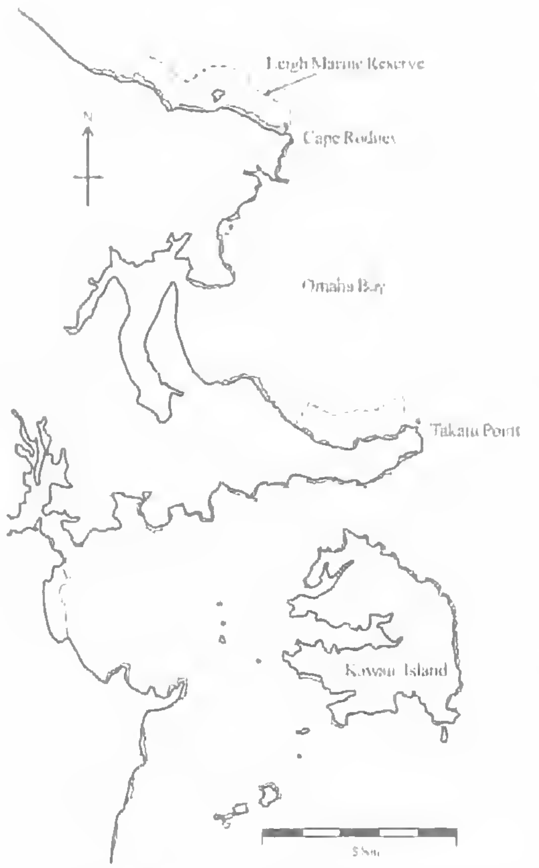

FIG. 1. Map showing the location of study sites in NE New Zealand, approximately 36°16'S, 174°48'E.

reported here is based on the approaches used by Pile et al. (1996, 1997), and investigates the following hypotheses: 1) That, like the temperate sponge *Mycale lingua* (Pile et al., 1996), *P. croceus* would efficiently consume large quantities of ultraplankton, in particular *Prochlorococcus* spp.; and 2) The rates at which water is processed by *P. croceus* would be high and relatively constant over diel periods. *Polymastia croceus* is a sponge capable considerable contraction, closing pores and withdrawing oscula, and for unknown reasons alternates between inflated and deflated forms. When deflated no oscula are visible and there is no apparent pumping activity taking place. In contrast, when inflated, large volumes of water appear to be turned over (confirmed visually using dye trace; Bell, 1998).

MATERIALS AND METHODS

Studies on diet and processing ability were undertaken at two sites on the NE coast of New Zealand where extensive *Polymastia croceus* biomass is found: Sponge Garden, within the Cape Rodney to Okakari Point (Leigh) Marine Reserve, and Takatu Point further to the south (Fig. 1). *Polymastia croceus* occurred between 16-18m below MLWS at both sites on sand covered base rock (Battershill & Bergquist, 1999b, in press).

DIET DETERMINATION. Flow cytometry was used to determine the diet of *P. croceus*. Following methods used by Pile (1997), five samples of ambient water (within 5cm of the sponge), and five samples of water being exhaled from oscula were taken *in situ* (using 5cc syringes) from each of five sponges at each site. Samples were fixed in 10% paraformaldehyde and frozen at -80°C, following the protocol described by Campbell et al. (1994). The samples were transported to Macquarie University (Sydney, Australia) for analysis of the ultraplankton composition of each sample using a FACScan Flow Cytometer unit (Becton Dickinson). The analysis technique was similar to that used by Marie et al. (1997). Two light scatter parameters were analysed: 1) forward light scatter, which relates to particle size; and 2) side light scatter, which relates to cell complexity. Three fluorescence parameters were also analysed: 1) green fluorescence from the SYBR Green 1 DNA stain (Molecular Probes Inc.); 2) orange fluorescence from the photopigment Phycoerythrin; and 3) red fluorescence from the photopigment Chlorophyll A. Each sample was run twice for all of these parameters. The first run was 100μl with autofluorescence being recorded, and the second was a 1 minute run of sample that had been stained with SYBR Green 1 (5μl SYBR Green 1 to 450μl sample).

The resultant data from the flow cytometry were then analysed using the custom designed Cytowin software (Vaulot, 1989), used to identify and enumerate the cells. Retention efficiency (RE) was determined by applying the following formula to each ultraplankton species:

$$RE=100\times(CCA-CCE)/CCA$$

where CCA is mean cell count ambient water, and CCE is mean cell count exhalant water.

TABLE 1. Summary of exhalant and ambient cell concentrations (mean number of cells ml-1 ± 1 SD) and resultant retention of ultraplankton species by *Polymastia croceus* at Sponge Garden. P-values for Students t-test, α=0.05.

Ultraplankton Species	Ambient ($x10^3$)	Exhalant ($x10^3$)	P-value	Percent Retained
Heterotrophic Bacteria	103.7 ± 37.7	55.8 ± 35.1	0.0000	46
Prochlorococcus spp.	20.5 ± 24.4	6.8 ± 4.1	0.0063	74
Synechococcus-type cyanobacteria	184.6 ± 26.6	11.7 ± 8.1	0.0000	94
Picoeukaryotes	8.1 ± 2.3	1.0 ± 0.4	0.0000	88

TABLE 2. Summary of exhalant and ambient cell concentrations (mean number of cells ml-1 ± 1 SD) and resultant retention of ultraplankton species by *Polymastia croceus* at Takatu Point. P-values for Students t-test, α=0.05.

Ultraplankton Species	Ambient ($x10^3$)	Exhalant ($x10^3$)	P-value	Percent Retained
Heterotrophic Bacteria	88.5 ± 28.7	70.8 ± 45.1	0.4759	20
Prochlorococcus spp.	13.1 ± 13.7	15.1 ± 24.2	0.3658	-15
Synechococcus-type cyanobacteria	117.0 ± 22.5	33.1 ± 30.0	0.0000	72
Picoeukaryotes	5.1 ± 2.3	1.8 ± 1.7	0.0000	65

PROCESSING RATE. To measure sponge pumping rates, a microthermistor-equipped datalogger was built for submarine use based on the design by Pile & Young (in prep.; modified from LaBarbera & Vogel, 1976). Colloquially known as a 'Medusa', the unit consists of a 12V battery, data logger and six microthermistor probes. The microthermistors were each placed over an osculum (five probes over oscula and one probe 20cm above the sponge in the ambient flow), and Fluorescein dye was used to visualise the outflow from the oscula to ensure that the probes were correctly in place perpendicular to the flow. The 'Medusa' was left in place for 24hr periods to log any changes in pumping rate over time. A Hobotemp temperature logger was also deployed, attached to the 'Medusa' housing, to enable the data to be calibrated for temperature variation. The logged data were down-loaded and calibrated with the temperature log data and calibration coefficients to allow conversion of voltage draw into flow rates of cm s^{-1}. All the sampled oscula were photographed and the images digitised to allow area measurements, which in turn permitted volume per unit time to be calculated.

RESULTS

DIET DETERMINATION. The most abundant ultraplankton (cells ml^{-1}) available to *Polymastia croceus* at both sites were *Synechococcus*-type cyanobacteria, followed by heterotrophic bacteria, *Prochlorococcus* spp. and autotrophic picoeukaryotes (Tables 1-2). Retention efficiencies, however, were highest for *Synechococcus*-type cyanobacteria followed by picoeukaryotes. At Sponge Garden (Table 1), retention efficiencies of *Prochlorococcus* spp. were next highest, followed by heterotrophic bacteria, while the opposite occurred at Takatu Point (Table 2). The absolute amounts of ultraplankton in ambient water differed between the two sites, although relative proportions were constant. Significant differences (one-way ANOVA with Bonferroni's pairwise comparisons, P<0.05) occurred between sites for the ambient concentrations of *Synechococcus*-type cyanobacteria, which averaged $184.6x10^3$ cells ml^{-1} at Sponge Garden but only $117.0x10^3$ cells ml^{-1} at Takatu Point. The ambient concentrations of the other species at Takatu Point were not significantly different from those at Sponge Garden (P>0.05). The retention efficiencies of sponges differed between the two sites with the mean retention of *Synechococcus*-type cyanobacteria, for example, being 94% at Sponge Garden and 72% at Takatu Point. The largest difference in retention occurred with *Prochlorococcus* spp.; 67% at Sponge Garden and -15% at Takatu Point. Differences between ambient and exhalant concentrations were tested (Students t-test, α=0.05) to confirm that the retention efficiencies were significant. Only heterotrophic bacteria and *Prochlorococcus* spp. at Takatu Point had insignificant differences.

PROCESSING RATE. Due to technical difficulties only two oscula had (at the time of writing), produced reliable results over a reasonable period (Fig. 2), but it is clear that *P. croceus* can pump at high velocities (Table 3). One oscule, for example, processed on average 26L an hour, or 304L an hour for every cm^2 of oscule area. The oscula showed a fairly constant pumping rate, with a period of heightened

TABLE 3. Summary of pumping rates (cm s^{-1}) of two oscula measured with the 'Medusa' at Sponge Garden. The estimates of volume pumped (cm^3 s^{-1}) were derived from the area of the oscule ($8.53mm^2$ for 1 and $6.96mm^2$ for 2).

Oscule	Measure	Average ± 1 SD	Min	Max
1	Velocity (cm s^{-1})	84.48 ± 4.78	80.15	103.44
2	Velocity (cm s^{-1})	64.14 ± 2.55	60.68	77.65
1	Volume (cm^3 s^{-1})	7.21 ± 0.41	6.84	8.82
2	Volume (cm^3 s^{-1})	4.45 ± 0.18	4.22	5.4

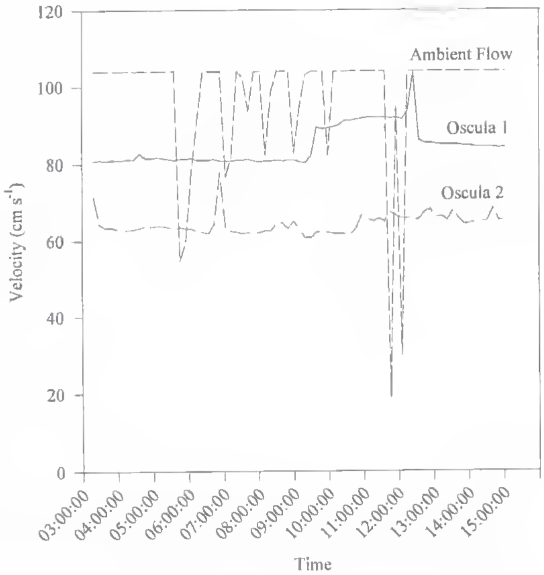

FIG. 2. Oscula velocity (cm s^{-1}) from *Polymastia croceus* at Sponge Garden. Velocity readings were taken at 10 minute intervals. Flow is a record of the ambient water velocity. The large spikes in the flow record are most likely the result of fish bites.

activity around midday hinting at periodicity in pumping rate. The sponges from which these results were derived were not fully inflated when studied, with only a few oscula per sponge open, and many of the surrounding sponges deflated. Thus, we believe these rates are likely to be conservative as an inflated sponge is likely to have greater pumping potential.

DISCUSSION

The hypothesis that *Polymastia croceus* would consume high percentages of ultraplankton, especially *Prochlorococcus* spp., proved to be partially correct. High percentages of ultraplankton were indeed consumed, but these consisted of *Synechococcus*-type cyanobacteria and picoeukaryotes as preferred dietary species, rather than *Prochlorococcus* spp., as reported in previous studies (Table 4). Heterotrophic bacteria and *Prochlorococcus* spp. were considerably less favoured, particularly at the Takatu Point site where their retention was statistically insignificant. The ambient samples showed that the most abundant ultraplankton species at both sites was *Synechococcus*-type cyanobacteria, correlating with it being the most retained species. However, picoeukaryotes, the least available ultraplankton species, had the second highest retention efficiency. This trend is most obvious at Takatu Point and suggests that a certain level of feeding selectivity by *P. croceus* may be present. Unselective feeding would be expected to show that those ultraplankton species occurring in higher numbers (cells ml^{-1}) in the water column would also be retained in proportionally higher numbers simply due to the higher probability of encounter. Although *Prochlorococcus* spp. appeared to be in higher abundance in exhalant water at Takatu Point, this was not significant and possibly an artefact of sampling, or due to the sponges concentrating patchily distributed *Prochlorococcus* spp. into their exhalant currents, with few or no *Prochlorococcus* spp. cells being taken up. *Synecococcus*-type cyanobacteria and picoeukaryotes are also smaller than heterotrophic bacteria or *Prochlorococcus* spp. and to extract them from the internal current especially at high velocities would be difficult. The general perception (e.g. Kilian, 1952; Bergquist, 1978; Pile, 1996) is that sponges are unselective filter feeders, whereas our findings that there is selectivity in dietary retention have implications for the distribution and abundance of *P. croceus* in the wild.

The Takatu Point sponges had lower retention of ultraplankton species (both in cells ml^{-1} and percent retained), than the sponges at Sponge Garden. The reasons for this can only be postulated at this stage, but there are three primary, interrelated possibilities. 1) Sponges may have a cycle of pumping, and the time of day at which they were sampled (mid-morning for Takatu Point and mid-afternoon for Sponge Garden), may be associated with different periods of pumping activity. 2) Sponges were inflated to different degrees at the different sampling sites. Inflation and deflation occurs in *P. croceus* in relation to unknown environmental conditions, which in itself may suggest that different microclimates exist at the two sites. The

TABLE 4. Comparison of ultraplankton retention efficiencies (%) and mean exhalant velocities between *Polymastia croceus* at Sponge Garden and previously studied species. Key: Hbac, heterotrophic bacteria; Pro, *Prochlorococcus* spp.; Syn, *Synechococcus*-type cyanobacteria; Peuks, autotrophic picoeukaryotes; 1, Pile et al. (1996); 2, Pile (1997); 3, Pile et al. (1997) with velocity for *B. bacilifera* from Savarese et al. (1997); 4, Reiswig (1971).

Sponge Species	Ultraplankton Species				Mean Exhalant Velocity (cm s^{-1})
	Hbac	Pro	Syn	Peuks	
Polymastia croceus	46	74	94	87	84.48 (SD = 4.78)
Ircinia felix [2]	30	26	48	-91	
Ircinia strobilina [2]	56	52	53	32	
Baikalospongia bacilifera [3]	71	NA	58	NA	4.3 (SD = 12.3)
Baikalospongia intermedia [3]	84	NA	66	99	
Mycale lingua [1]	74	93	89	86	14 (SD = 9.7)
Mycale sp. [4]	NA	NA	NA	NA	7.8
Tethya crypta [4]	NA	NA	NA	NA	15

Takatu Point sponges were observed to be less 'open' than those at Sponge Garden, so they may have been going into, or coming out of, a period of deflation and thus processing at a lower, less efficient rate. The lower cells ml^{-1} available at Takatu Point may be linked to the suppressed pumping/retention activity, as it is possible that food availability may be a cue for inflation/deflation (pers. observations). 3) The Takatu Point sponges, which are positioned adjacent to a constant long shore current, have some other nutrient source which can be, for example, absorbed through the pinacoderm making filtering for food less necessary. Other studies (e.g. Kilian, 1952) have shown that particles can be ingested by the pinacoderm and thus direct uptake of nutrients from the ambient water is possible, although unlikely to be significant to the sponge's nutritional requirements.

In situ sampling provided a realistic insight into the feeding ecology of sponges, although this insight is still limited given the lack of temporal and spatial variation in sampling. Certainly, differences between the two sites sampled suggest that there may be considerable spatial variation. The inference of selectivity shown at Takatu Point is something that has yet to be demonstrated in sponges and is an exciting find, but its verification requires considerably more work.

We hypothesised that *P. croceus* would have relatively high pumping rates that were fairly constant over time. This cannot be verified due to the lack of data, but we can confirm the average flow velocities of 84.48cm s^{-1} and 64.14cm s^{-1} are high compared to those found by Reiswig (1971), Pile et al. (1996) and Savarese et al. (1997) (Table 4). It was not possible to determine mean pumping rates for *P. croceus* per unit biomass, a relationship which would have allowed more relevant comparisons to be made with data from the literature. As the sponges were not fully inflated at the time, pumping rates — particularly those in relation to sponge biomass — were likely to have been depressed. Variations in pumping velocities over time suggest that there may be periods of increased velocity which are independent of the ambient flow. Previous studies confirm that pumping rates can vary over time, although this appears to be species dependant. *Mycale* sp., for example, maintained a fairly constant level of water transport, whereas *Tethya crypta* had a highly changeable pumping rate, apparently determined by light intensity (Reiswig, 1971). Savarese et al. (1997) also noted high variability in pumping rates over time and with location for the freshwater sponge *Baikalospongia*. While the results for *P. croceus* are far from conclusive, it is possible that there was some variability in pumping over short periods of time. The inflation/deflation phenomenon certainly shows that over longer periods of time there is large variation in the water processing potential of any given biomass. Further work with the 'Medusa' would allow both short- and long-term variability to be better defined, and thus enhance the potential for making predictions on the amount of water that can be turned over in any given period of time.

Determination of the variability in pumping performance with site would allow some gauge of the influence of environmental factors on pumping performance. Variation in ambient flows between sites may show the extent to which flow can assist pumping, and whether this assistance is related to differences between sites, such as the distribution and biomass of sponges: in other words, whether or not assisted flow enhances the growth and distribution of *P. croceus*. Ecological studies (Bell, 1998) have provided estimations of pumping rates per unit biomass during an inflation (and thus maximum pumping) event. The calculated mean oscula area per m^2 at Sponge Garden, when combined with the mean velocity from oscule 2 (64.14cm s^{-1}), produced an estimate of 54ml s^{-1} m^{-2}. This estimated rate is probably conservative, but its extrapolation suggests that the water column directly above the sponges is turned over approximately every 9.3hrs at Sponge Garden.

In summary, *P. croceus* appears to be able to process large volumes of water over short periods. This could lead to extremely high rates of carbon consumption by this species, which has potentially significant implications for the benthic marine ecosystem. For example, removing or adding *P. croceus* to habitats such as during harvesting or aquaculture ventures, would impact significantly on these habitats, particularly in terms of the availability of primary production.

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